ROS Brite™ 570 *Optimized for Detecting Reactive Oxygen Species (ROS)*

Catalog number: 16000
Unit size: 1 mg

Component	Storage	Amount (Cat No. 16000)
ROS Brite™ 570 *Optimized for Detecting Reactive Oxygen Species (ROS)*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include superoxide, hydroxyl radical, singlet oxygen and peroxides. ROS is highly reactive due to the presence of unpaired valence shell electrons. ROS forms as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation. Under conditions of oxidative stress, ROS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids. Oxidative damage of these biomolecules is associated with aging as well as with a variety of pathological events, including atherosclerosis, carcinogenesis, ischemic reperfusion injury, and neurodegenerative disorders. ROS Brite™ 570 reagent is a new fluorogenic probe to measure oxidative stress in cells using conventional fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The cell-permeant ROS Brite™ 570 reagent is nonfluorescent and produces bright orange fluorescence upon ROS oxidation. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. It is an excellent alternative to CellROX™ Orange Reagent (C10443) for oxidative stress detection (CellROX™ is a trademark of ThermoFisher).

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

ROS Brite™ stock solution

Prepare a 10 to 20 mM ROS Brite™ stock solution in DMSO.

Note: The stock solution can be stored at -20° C in single use aliquotes. Protect from light.

PREPARATION OF WORKING SOLUTION

ROS Brite™ working solution

Make 5 to 10 µM working solution by diluting the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).

Note: The working solution should be made fresh before use.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells as desired (e.g., RASM cells are treated with 50-100 nM angiotensin II for 3-5 hours).
2. Incubate the cells with ROS Brite™ (5-10 µM) for 15 -30 minutes at 37°C.

   Note: The concentration of ROS Brite™ used varies with different cell lines, one will need test with different concentrations to get the optimal dose.
3. Replace the working solution with HHBS buffer.
4. Analyze the cells with a proper fluorescence instrument (e.g., a fluorescence microscope, flow cytometer or an in vivo imaging instrument).

EXAMPLE DATA ANALYSIS AND FIGURES

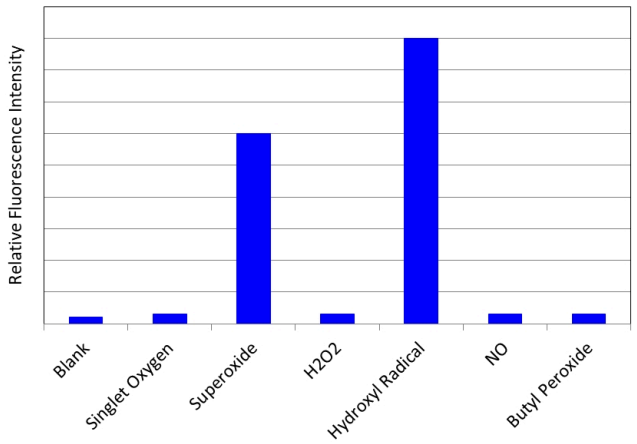


Figure 1. Fluorescence response of ROS Brite™ 570 to different reactive oxygen species in PBS buffer (pH 7.2). The fluorescence intensities were measured with Ex/Em = 540/590 nm.

DISCLAIMER

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